



# Sex Differences in the Hepatotropic Effects of Antiulcer Drugs and Placenta Cryoextract in an Experimental Rat Liver Injury Model

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## Abstract

**Background/Aim:** Sex-related variances in drug metabolism provide a foundation for refining treatment protocols for prevalent conditions based on the patient's sex. Tailoring treatment strategies based on sex is particularly noteworthy among patients with comorbid illnesses due to the potential for drug interactions and the impact of concurrent diseases on clinical outcomes. Aim of this study was to assess the hepatotropic effects of antiulcer drugs (esomeprazole, clarithromycin and metronidazole – E/C/M) and placenta cryoextract (CEP) within a simulated model of tetrachloromethane (CCl<sub>4</sub>)-induced hepatitis combined with underlying ethanol-induced liver cirrhosis (EILC), with a focus on the role of subjects' sex.

**Methods:** Using 112 male and female rats, the research explored the effects of different sex hormone levels. Chronic EILC was induced by administering a 50.0 % CCl<sub>4</sub> oil solution (8 mL/kg) twice a week, combined with a 5.0 % ethanol solution, over 45 days. Total protein (TP) levels and alkaline phosphatase (AP) activity were measured spectrophotometrically.

**Results:** The research findings indicate that the onset of EILC and the administration of E/C/M resulted in a significantly greater 10.8 % ( $p = 0.03$ ) reduction in TP levels among females compared to males, without altering hormonal status. Introducing CEP led to a noteworthy ( $p < 0.001$ ) rise in TP levels, by 30.8 % in males and 33.9 % in females, in the context of EILC and E/C/M administration, while maintaining hormonal status.

Among male rats, the most elevated AP activity was observed with excess testosterone propionate administration (5.0 [5.0; 5.9]  $\mu\text{mol/L}$ ), while the lowest level was recorded in rats after testectomy, measuring 3.8 [2.5; 4.7]  $\mu\text{mol/L}$ , exhibiting a significant 20.8 % decrease ( $p < 0.05$ ) compared to male rats without hormonal status changes. In female rats, the study revealed that against the backdrop of EILC and E/C/M administration, the highest AP level was seen in ovariectomised females, reaching 5.8 [5.1; 6.2]  $\mu\text{mol/L}$ , reflecting a substantial 9.4 % increase compared to rats without hormonal status changes.

**Conclusions:** The administration of CEP under similar experimental conditions led to the recovery of the liver's protein-synthesising function in both male and female rats. When female sex hormones were introduced to sham-operated female rats, a significant 20.8 % greater reduction in AP levels was observed. Additionally, gonadectomy led to a more pronounced decrease in this enzyme's levels in male rats compared to female rats, indicating the cytoprotective properties of female sex hormones.

**Key words:** Cryopreserved placenta extract; Peptic ulcer; Hepatitis; Liver cirrhosis; Sexual dimorphism; Comorbidity.

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## Introduction

Considering sex-based disparities in the pharmacodynamics and pharmacokinetics of medicinal products (MPs) is a critical factor in achieving effective pharmacological treatment. Both endogenous and exogenous sex hormones can directly or indirectly impact the metabolism of MPs. Additionally, certain drugs possess the potential to induce changes in hormonal signalling pathways.<sup>1</sup> The oxidative biotransformation of medicinal products in the liver, mediated by a series of cytochrome P450 (CYP) isoenzymes, plays a crucial role in the therapeutic effectiveness of these products.<sup>1,2</sup> Individual variations in the expression of key enzymes involved in drug

metabolism, including CYP P450, sulfotransferases, glutathione transferases and glucuronosyltransferases are linked to significant individual differences in the bioavailability and clearance of drugs and other xenobiotics. Given the central role of hepatic enzymes in regulating the pharmacological and biological activity of drugs, as well as steroids and other endobiotics, it is imperative to comprehend the regulatory characteristics that contribute to individual disparities in their expression.<sup>3</sup> It is noteworthy that the most crucial isoform of CYP in drug metabolism, CYP 3A4, exhibits higher expression levels in the liver of women as compared with men.<sup>4,5</sup>

**Table 1:** Biologically active substances present in placental cryoextract<sup>#0, 11</sup>

Biologically active substances	Properties	Range values
$\alpha$ -fetoprotein	Growth regulator of embryonic, transformed, activated immune competent cells	429 $\pm$ 75 mIU/mL
Chorionic gonadotropin	Immune system activator, promotes the production of steroid hormones (testosterone and oestradiol)	26.8 $\pm$ 8 mIU/mL
Estradiol	Reproductive function, cardioprotective action	755 $\pm$ 48 pmol/mL
Progesterone	Reproductive function, cardioprotective action	226 $\pm$ 110 nmol/mL
Prolactin	Impact on the development of secondary sexual characteristics, erythropoietic action, regulation of lipid metabolism	705 $\pm$ 129 mIU/mL
Fertility $\alpha$ -microglobulin	Readiness for pregnancy, the conception process and the normal development of the foetoplacental unit	1470 $\pm$ 173 ng/mL
Lactoferrin	Stimulation of lactation	1270 $\pm$ 223 ng/mL
Somatotropin	Growth hormone, anabolic action	5.64 ng/mL
Luteinising hormone	Pituitary hormone, secretion of oestrogens, progesterone, testosterone	7.8 $\pm$ 1.9 IU/L
Follicle-stimulating hormone	Pituitary hormone, promotes follicle maturation in ovaries and spermatogenesis	7.1 $\pm$ 2.3 mIU/L
Testosterone	Differentiation and functioning of the reproductive system, anabolic action	3.68 $\pm$ 1.06 nmol/mL
Thyroid-stimulating hormone	Stimulation of thyroid function, immunomodulatory action	291 $\pm$ 13 mIU/L
Triiodothyronine	Stimulation of metabolism, growth and tissue differentiation, reproduction, haematopoiesis	2.1 $\pm$ 0.6 pmol/L
Thyroxine	Stimulation of metabolism, growth and tissue differentiation, reproduction, haematopoiesis	5.6 $\pm$ 0.99 pmol/L
Cortisol	Metabolism of proteins, carbohydrates, fats and nucleic acids	1392 $\pm$ 515 nmol/mL
Colony-stimulating factor	Proliferation of bone marrow cells	9.87 ng/mL
Tumour necrosis factor- $\alpha$	Inhibitor of cancer cell proliferation	84.5 pg/mL
Interleukin 1 $\beta$	Regulation of pluripotent stem cell differentiation and the immunendocrine system	201.7 pg/mL
Interleukin 4	Regulation of pluripotent stem cell differentiation and the immunendocrine system	21.7 pg/mL
Interleukin 6	Regulation of pluripotent stem cell differentiation and the immunendocrine system	114.9 pg/mL
Total protein	Plastic function	76.5 $\pm$ 14.0 mg/L g of tissue
Proteins with a molecular mass of 20–100 kDa	Plastic function	70–80 %
Proteins with a molecular mass below 20 kDa	Plastic function	20–30 %

As a potential agent capable of mitigating the hepatotoxic effects of medicinal products while exhibiting its own anti-ulcer activity, attention of authors was drawn to placental cryoextract (CEP). This cryoextract was developed and introduced into practice by scientists from the Institute of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (Institute), who have also devised a methodology for its long-term storage in a low-temperature environment.<sup>6,7</sup> The placenta serves as a natural depot and producer of a wide range of biologically active substances (Table 1), which facilitate the growth and development of the foetus during intrauterine development. It supports processes such as trophic interactions and protein synthesis, gas exchange, hormone secretion, blood pressure regulation, blood clotting, detoxification, metabolite excretion, deposition of biologically active substances, immune regulation, regulation of lipid peroxidation and more.<sup>8-10</sup>

The aim of the study was to characterise the sex-specific features of the hepatotropic effects of anti-ulcer agents and placental cryoextract in the context of experimental chronic liver injury.

## Methods

The experimental research was conducted on 112 male and female rats weighing 200–220 g, divided into four groups of 28 animals each: Group I (males) and Group III (females) – rats with simulated tetrachloromethane (CCl<sub>4</sub>)-induced hepatitis alongside ethanol-induced liver cirrhosis. These rats were subjected to daily intragastric administration for 7 days of esomeprazole (50 mg/kg), clarithromycin (91 mg/kg) and metronidazole (91 mg/kg) (E/C/M);<sup>12, 13</sup> Group II (males) and Group IV (females) consisted of rats with simulated CCl<sub>4</sub>-induced hepatitis alongside ethanol-induced liver cirrhosis. These rats were subjected to daily intragastric administration of E/C/M for 7 days following the same scheme as described earlier. Additionally, from the 3rd to the 7th day of administration of anti-ulcer agents (5 administrations), placental cryoextract (CEP) was introduced (0.16 mg/kg, intramuscularly). Each group comprised 4 subgroups with varying hormonal statuses, each containing 7 animals:

Subgroup a) – Rats of both sexes that underwent sham surgery and received replacement hormone therapy (excess).

Subgroup b) – Rats of both sexes that underwent sham surgery without a change in hormonal status (comparison group).

Subgroup c) – Rats of both sexes that underwent testectomy or ovariectomy.

Subgroup d) – Rats of both sexes that, after gonadectomy, received replacement hormone therapy.

The animals were removed from the experiment 24 hours after the last administration of CEP using cervical dislocation under inhalation anaesthesia.

### Modelling experimental pathology

Chronic CCl<sub>4</sub>-induced hepatitis with concurrent ethanol-induced liver cirrhosis (EILC) was replicated through intragastric administration of a 50.0 % oil solution of CCl<sub>4</sub> at a dose of 8 mL/kg animal body weight, twice a week, combined with a 5.0 % ethanol solution for drinking over a period of 45 days.<sup>13, 16</sup>

Modulation of sex hormone levels was achieved through surgical ovariectomy or testectomy in female and male rats, respectively, using established methods.<sup>13-15</sup> The investigations were carried out 21 days after gonadectomy. Untreated animals in the control groups underwent an incision of the anterior abdominal wall followed by wound closure (sham-operated animals). Substitution and excessive hormone therapy were conducted for 14 days in males by subcutaneous administration of testosterone propionate (*PAT Pharmak*, Ukraine) at a dose of 1 mg/kg once a day and in females by intragastric administration of oestradiol hemihydrate (*Abbott Biologicals* BV, Netherlands) at a dose of 150 mg/kg.<sup>17-22</sup> CEP was obtained from the Interdepartmental Scientific Centre of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, National Academy of Medical Sciences and Ministry of Health of Ukraine, in the form of an ampoule product named *Cryocell – placenta cryoextract*.

### Biochemical research methods

The study material consisted of serum from whole peripheral blood. Samples of mixed blood were collected in centrifuge tubes after decapita-

tion of the animals. Serum was separated by centrifugation for 15 min at 3000 rpm.

The content of total protein (TP) was determined using a spectrophotometric method based on the biuret reaction. In this method, under alkaline conditions, divalent copper ions ( $\text{CuSO}_4$ ) react with proteins to form a violet-coloured complex. The protein concentration was measured spectrophotometrically by light absorption at a wavelength of  $\lambda = 546$  nm and expressed in g/L.<sup>23</sup>

The activity of alkaline phosphatase (AP) was determined using a spectrophotometric method based on the property of AP to hydrolyse the ether bond in  $\beta$ -glycerophosphate, releasing phosphoric acid. The generated phosphate content was determined using a reaction with a molybdenum reagent in the presence of ascorbic acid. The intensity of the resulting molybdenum blue coloration is proportional to the amount of phosphate.<sup>24</sup>

### Bioethical aspects of the study

All experimental research on laboratory animals followed Good Laboratory Practice standards, as outlined in "Medicinal Products. Good Laboratory Practice," approved by the Ministry of Health

of Ukraine. The research also adhered to the Council of Europe Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Directive 2010/63/EU and relevant Ukrainian laws. The comprehensive research program was approved by the Bioethics Committee at the Institute (Protocol No 2, 3 January 2022; Protocol No 5, 22 November 2022).

### Statistical analysis

The distribution of variables within each group was assessed using the Shapiro-Wilk test. Variance homogeneity was examined through Levene's test. For normally distributed independent variables, pairwise group differences were analysed using Student's t-test and ANOVA with Fisher's parametric F-test. Non-normally distributed data comparisons utilised the non-parametric Mann-Whitney rank test and Kruskal-Wallis rank-based analysis. Normally distributed data was presented as " $M \pm m$ " ( $M \pm SE$ ), where M represents the mean and m (SE) corresponds to the standard error of the mean, along with a 95 % confidence interval (95 % CI). Non-normally distributed data were denoted as Me [LQ; UQ], where Me indicates the median and [LQ; UQ] signifies the upper and lower quartile bounds.<sup>25</sup>

## Results

The study revealed that the progression of  $\text{CCl}_4$ -induced hepatitis and the administration of E/C/M resulted in a 10.8 % greater ( $p = 0.03$ ) decrease in TP levels in females compared to males without a change in hormonal status (Table 2). The administration of testosterone propionate led to a statistically significant ( $p < 0.01$ ) 12.2 % greater decrease in TP levels in male rats with  $\text{CCl}_4$ -induced hepatitis in the presence of E/C/M. In contrast, in castrated males, TP levels were 11.0 % higher than those of rats without a change in hormonal status (Table 3). Notably, ovariectomy in females with  $\text{CCl}_4$ -induced hepatitis in the context of E/C/M administration resulted in a 11.6 % decrease ( $p = 0.01$ ) in TP levels in peripheral blood compared to females without a change in hormonal status.

Administration of CEP resulted in a statistically significant ( $p < 0.001$ ) increase in TP levels by 30.8 % in males and 33.9 % in females without

a change in hormonal status, in the context of  $\text{CCl}_4$ -induced hepatitis and E/C/M administration (Table 3). Notably, the smallest increase in TP levels following CEP administration was observed in females receiving excess oestradiol hemihydrate, which was in line with the highest levels of the investigated parameter in female rats not receiving CEP, at  $54.1 \pm 1.30$  g/L and  $70.4 \pm 1.32$  g/L, respectively. These findings suggest a protective role of female sex hormones in liver toxic injuries. Conversely, in male rats with  $\text{CCl}_4$ -induced hepatitis, E/C/M administration, excess testosterone propionate and CEP, the most significant increase in TP levels was observed ( $p < 0.001$ ). Meanwhile, male rats not receiving CEP showed the lowest TP levels, at  $47.9 \pm 1.56$  g/L (Table 2). The obtained data indicate the ability of CEP to restore liver protein synthesis function in both male and female rats with  $\text{CCl}_4$ -induced hepatitis, in the context of E/C/M administration.

**Table 2:** Effect of CEP and E/C/M on the serum total protein content in chronic ethanol-tetrachloromethane-induced liver injury in male and female rats, g/L ( $M \pm m$  (95 % CI),  $n = 112$ )

The study parametre	Group	Males		Females	
		Group I	Group II	Group III	Group IV
		EILT + E/C/M 7*	EILT + CEP + E/C/M 7*	EILT + E/C/M 7*	EILT + CEP + E/C/M 7*
Without alterations to the hormonal status	a	54.60 ± 1.72 (95 % CI: 51.20–57.90)	71.40 ± 1.86 (95 % CI: 67.80–75.10)	49.30 ± 1.38 (95 % CI: 46.60–52.00)	66.00 ± 1.91 (95 % CI: 62.20–69.80)
		$p_{1-2} < 0.001$		$p_{1-3} = 0.030$ $p_{2-3} < 0.001$	$p_{1-4} < 0.010$ $p_{2-4} = 0.600$ $p_{3-4} < 0.001$
Hormone therapy	b	47.90 ± 1.56 (95 % CI: 44.80–50.90)	65.90 ± 2.60 (95 % CI: 60.80–71.00)	54.10 ± 1.30 (95 % CI: 51.60–56.70)	70.40 ± 1.32 (95 % CI: 57.80–73.00)
		$p_{a-b} = 0.190$	$p_{a-b} = 0.110$ $p_{1-2} < 0.001$	$p_{a-b} = 0.020$ $p_{1-3} < 0.010$ $p_{2-3} < 0.010$	$p_{a-b} = 0.080$ $p_{1-4} < 0.001$ $p_{2-4} = 0.140$ $p_{3-4} < 0.001$
Gonadectomy with hormonal replacement therapy	c	58.30 ± 2.01 (95 % CI: 54.30–62.20)	73.70 ± 3.03 (95 % CI: 67.80–79.70)	45.60 ± 1.60 (95 % CI: 42.40–48.70)	62.40 ± 1.19 (95 % CI: 60.10–64.80)
		$p_{a-c} = 0.190$ $p_{b-c} < 0.010$	$p_{a-c} = 0.530$ $p_{1-2} < 0.010$ $p_{b-c} = 0.070$	$p_{a-c} = 0.100$ $p_{1-3} < 0.001$ $p_{b-c} < 0.010$ $p_{2-3} < 0.001$	$p_{a-c} = 0.140$ $p_{1-4} = 0.100$ $p_{b-c} < 0.001$ $p_{2-4} < 0.010$ $p_{3-4} < 0.001$
Gonadectomy	d	60.60 ± 3.84 (95 % CI: 53.10–68.10)	74.60 ± 6.19 (95 % CI: 62.40–86.70)	43.60 ± 1.32 (95 % CI: 41.00–46.20)	58.70 ± 2.86 (95 % CI: 53.10–64.60)
		$p_{a-d} = 0.180$ $p_{b-d} < 0.010$ $p_{c-d} = 0.610$	$p_{a-d} = 0.640$ $p_{1-2} = 0.080$ $p_{b-d} = 0.220$ $p_{c-d} = 0.900$	$p_{a-c} = 0.100$ $p_{1-3} < 0.001$ $p_{b-c} < 0.010$ $p_{2-3} < 0.001$	$p_{a-d} = 0.160$ $p_{1-4} = 0.700$ $p_{b-d} < 0.010$ $p_{2-4} = 0.040$ $p_{c-d} = 0.250$ $p_{3-4} < 0.001$

Notes. Indices  $_{1,2,3,4}$  indicate the group number depending on the investigated drugs, between the indicators of which a comparison has been conducted; Indices a, b, c, d indicate the group number depending on the hormonal status, between the indicators of which a comparison has been performed;  $p_{2-1}$  – the level of statistical significance of the difference between the groups; CEP, cryoextract of placenta; CI, confidence interval; E/C/M, esomeprazole, clarithromycin and metronidazole; EILT, ethanol-induced liver cirrhosis; HRT, hormonal replacement therapy; \*: number of animals in group;

**Table 3:** Effect of E/C/M and HRT on alkaline phosphatase activity in blood serum in chronic ethanol-tetrachloromethane-induced liver injury in male and female rats, g/L ( $M \pm m$  (95 % CI) or Median [interquartile range],  $n = 112$ )

The study parametre	Group	Males		Females	
		Group I	Group II	Group I	Group II
		EILT + E/C/M 7*	EILT + CEP + E/C/M 7*	EILT + E/C/M 7*	EILT + CEP + E/C/M 7*
Without alterations to the hormonal status	a	4.80 ± 0.10 (95 % CI: 4.60–5.00)	2.60 ± 0.09 (95 % CI: 2.40–2.70)	5.30 ± 0.27 (95 % CI: 4.80–5.80)	2.70 ± 0.12 (95 % CI: 2.40–2.90)
		$p_{1-2} < 0.001$		$p_{1-3} = 0.100$ $p_{2-3} < 0.001$	$p_{1-4} < 0.010$ $p_{2-4} = 0.510$ $p_{3-4} < 0.001$
Hormone therapy	b	5.0 [5.0; 5.9]	3.0 [2.5; 3.4]	5.1 [4.5; 5.3]	2.0 [1.8; 2.7]
		$p_{a-b} = 0.03$	$p_{a-b} = 0.080$ $p_{1-2} < 0.001$	$p_{a-b} = 0.260$ $p_{1-3} = 0.140$ $p_{2-3} < 0.010$	$p_{a-b} = 0.060$ $p_{1-4} < 0.001$ $p_{2-4} = 0.020$ $p_{3-4} < 0.001$
Gonadectomy with hormonal replacement therapy	c	4.10 ± 0.23 (95 % CI: 3.70–4.60)	2.20 ± 0.14 (95 % CI: 1.90–2.50)	5.20 ± 0.15 (95 % CI: 4.90–5.50)	3.10 ± 0.11 (95 % CI: 2.90–3.30)
		$p_{a-c} = 0.020$ $p_{b-c} < 0.010$	$p_{a-c} = 0.030$ $p_{1-2} < 0.001$ $p_{b-c} < 0.010$	$p_{a-c} = 0.100$ $p_{1-3} < 0.001$ $p_{b-c} < 0.010$ $p_{2-3} < 0.001$	$p_{a-c} = 0.010$ $p_{1-4} < 0.010$ $p_{b-c} < 0.010$ $p_{2-4} < 0.001$ $p_{3-4} < 0.001$
Gonadectomy	d	3.8 [2.5; 4.7]	2.0 [1.7; 2.5]	5.8 [5.1; 6.2]	3.5 [3.5; 3.7]
		$p_{a-d} < 0.050$ $p_{b-d} < 0.010$ $p_{c-d} = 0.330$	$p_{a-d} = 0.090$ $p_{1-2} < 0.010$ $p_{b-d} = 0.020$ $p_{c-d} = 0.420$	$p_{a-d} = 0.240$ $p_{1-3} = 0.010$ $p_{b-d} = 0.030$ $p_{2-3} < 0.001$ $p_{c-d} = 0.080$	$p_{a-d} < 0.001$ $p_{1-4} = 0.400$ $p_{b-d} < 0.001$ $p_{2-4} < 0.001$ $p_{c-d} = 0.010$ $p_{3-4} < 0.001$

Notes. Indices  $_{1,2,3,4}$  indicate the group number depending on the investigated drugs, between the indicators of which a comparison has been conducted; Indices a, b, c, d indicate the group number depending on the hormonal status, between the indicators of which a comparison has been performed;  $p_{2-1}$  – the level of statistical significance of the difference between the groups; CEP, cryoextract of placenta; CI, confidence interval; E/C/M, esomeprazole, clarithromycin and metronidazole; EILT, ethanol-induced liver cirrhosis; HRT, hormonal replacement therapy; \*: number of animals in group;



In the context of E/C/M administration and the development of  $\text{CCl}_4$ -induced hepatitis, there was a parallel increase in AP levels in peripheral blood in both male and female rats, reaching 4.8  $\mu\text{mol/L}$  and 5.3  $\mu\text{mol/L}$ , respectively (Table 3). Male rats exhibited their highest AP activity levels in response to excessive testosterone propionate administration (5.0 [5.0; 5.9]  $\mu\text{mol/L}$ ), while the lowest levels were observed in rats after testectomy, registering at 3.8 [2.5; 4.7]  $\mu\text{mol/L}$ . This difference was statistically significant ( $p < 0.05$ ), with a reduction of 20.8 % compared to male rats without hormonal alterations (Table 3). These findings underscore the potential of male sex hormones to amplify destructive processes, especially within the liver. The study revealed that female rats, in the context of  $\text{CCl}_4$ -induced hepatitis progression and E/C/M administration, displayed the highest AP activity level among rats that had undergone ovariectomy, measuring 5.8 [5.1; 6.2]  $\mu\text{mol/L}$ . This level outpaced that of rats without hormonal changes by 9.4 %.

The introduction of CEP led to a significant reduction in AP activity levels in both male and female rats. Female rats with  $\text{CCl}_4$ -induced hepatitis who received E/C/M, oestradiol hemihydrate and CEP exhibited the most substantial decrease in AP activity. This parameter showed a marked statistical reduction ( $p < 0.001$ ), dropping by 60.8 % compared to female rats not administered CEP (Table 3). Among female rats with CEP administration, the least reduction in AP activity was observed in rats after ovariectomy, with a decrease of 39.7 % compared to the group without CEP administration ( $p < 0.001$ ). Among male rats, during  $\text{CCl}_4$ -induced hepatitis and E/C/M administration, as well as CEP treatment, the AP activity level decreased by an average of 44.9 % (ranging from 40.0 % during excessive hormonal therapy to 47.4 % during gonadectomy), compared to rats without CEP administration (Table 3).

## Discussion

The development of  $\text{CCl}_4$ -induced hepatitis is known to be associated with a reduction in the TP level in peripheral blood, which is due to impaired liver protein synthesis function.<sup>35</sup> The study revealed that the progression of  $\text{CCl}_4$ -induced hepatitis and the administration of E/C/M resulted in a 10.8 % greater ( $p = 0.03$ ) decrease in TP levels in females compared to males without a change in hormonal status.

To assess destructive processes in liver tissues, the study examined the AP activity in peripheral blood, as this enzyme is present, among other places, in the walls of liver bile ducts and reflects their integrity. It was observed that in the context of E/C/M administration and the development of  $\text{CCl}_4$ -induced hepatitis, there was a parallel increase in AP levels in peripheral blood in both male and female rats, reaching 4.8  $\mu\text{mol/L}$  and 5.3  $\mu\text{mol/L}$ , respectively. This elevated AP activity suggests the development of cholestasis, which is consistent with existing literature findings.<sup>36</sup>

Freire et al<sup>2</sup> have comprehensively examined gender-related differences in gastrointestinal tract (GIT) functionality. Their study reveals that stomach pH tends to be higher in women than in men, whereas the transit time of chyme through the stomach and intestines is comparatively shorter. Notably, certain disparities are associated with progesterone and oestrogen levels, which are influenced by menstrual cycle phases or pregnancy. The interplay of pH in the lumen and GIT motility significantly affects drug bioavailability, impacting both drug degradation rates and transit times. This dynamic, for instance, can result in an extended postprandial waiting period for drug intake.

Considering gender differences in drug metabolism and the physiological characteristics of the GIT between men and women, the optimisation of treatment regimens for the most prevalent digestive system disorders, taking into account sex hormone levels, becomes a pivotal goal in contemporary gastroenterology and hepatology.<sup>3,4</sup> Of particular interest is the gender-based differentiation of treatment approaches in patients with comorbid conditions, due to the risk of interactions between drugs and the influence of concomitant diseases on clinical outcomes. In patients with liver diseases, the most common comorbid conditions among others include arterial hypertension, oesophagitis, dyslipidaemia, diabetes mellitus and peptic ulcer disease (PUD).<sup>26</sup> According to Kim et al,<sup>27</sup> the prevalence of PUD among cirrhosis patients is reported to be 24.3 %, while the prevalence of *Helicobacter pylori* infection among patients with virus-induced liver cirrhosis (42.5 %) is significantly higher than among alcohol-induced liver cirrhosis patients (22.0 %,  $p < 0.001$ ). In their examination of 619 outpatient patients with liver cirrhosis and 142 healthy control subjects, Saboo et al<sup>28</sup> in a study conducted in 2021, found that a greater proportion of liver cirrhosis patients were taking proton pump inhibitors

(PPIs) as compared with the control group ( $p < 0.0001$ ) and more men than women were using PPIs. However, it's noteworthy that PPIs, while reducing stomach acid levels, can decrease the bioavailability of medications that require intra-gastric acidity to maximise their absorption and bioavailability.<sup>29</sup>

The need to use medications from different pharmacological groups in patients with chronic liver diseases accompanied by PUD heightens the risks of pharmacodynamic interactions and the development of undesirable side effects, particularly hepatotoxicity associated with medications used to treat peptic ulcers.<sup>30-34</sup>

Presented study underscores the significant influence of sex hormones on the development of  $\text{CCl}_4$ -induced hepatitis and the response to this condition. In general,  $\text{CCl}_4$ -induced hepatitis is accompanied by a decrease in TP levels in the blood, indicating impaired liver protein synthesis function. The results reveal gender-specific differences in the response to hepatitis and hormone administration. Females experience a 10.8 % greater decrease in TP levels compared to males when E/C/M is administered, even in the absence of hormonal changes. Conversely, the administration of testosterone propionate to male rats with  $\text{CCl}_4$ -induced hepatitis in the presence of E/C/M results in a substantial 12.2 % greater decrease in TP levels, highlighting the potential negative impact of excessive male sex hormones. Castrated males, however, show an 11.0 % increase in TP levels compared to male rats without hormonal changes, indicating a potentially protective effect of removing male sex hormones. Ovariectomy in females with  $\text{CCl}_4$ -induced hepatitis and E/C/M administration leads to an 11.6 % decrease in TP levels compared to females without hormonal changes. As a result, research showed that sex hormones, particularly testosterone and oestradiol, have a significant impact on the progression of  $\text{CCl}_4$ -induced hepatitis and the therapeutic response to CEP. The results suggest a potential protective role of female sex hormones and a detrimental effect of excessive testosterone. Moreover, CEP appears to have a positive influence on TP levels and AP activity, potentially ameliorating the liver damage caused by hepatitis. Further research and a deeper understanding of these interactions are essential for the development of effective treatments for liver diseases.

## Conclusion

1. The combined use of anti-ulcer drugs in the context of  $\text{CCl}_4$ -induced hepatitis displayed gender-determined differences. Specifically, female rats without hormonal alterations exhibited lower TP levels. Administering CEP under analogous experimental conditions led to the restoration of liver protein synthesis function in rats of both genders.

2. Among female rats subjected to sham surgery, a 20.8 % greater reduction in AP levels was observed when exposed to female sex hormones, whereas gonadectomy resulted in a more pronounced decrease in this enzyme's levels in male rats compared to female rats. This suggests the cytoprotective properties of female sex hormones.

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## Conflict of interest

None.

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